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Yuko Ichihara

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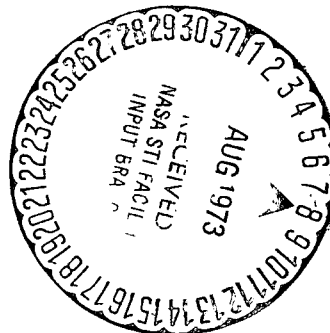
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| 16. Abstract The study clarifies the concentration, composition and other aspects of amino acids present in the Cenozoic formations of Japan. The samples used were mainly muddy samples, collected from representative Cenozoic formations in Japan. Various conclusions could be drawn: 1) The amino acid concentration diminishes as the geological age of the formation gets older. 2) Amino acid concentration is high in clayey and low in sandy sediments. 3) Nineteen kinds of amino acids were detected, 17 of which were α -amino acids. 4) The amino acid composition percentage is similar in all the samples. 5) It is supposed that amino sugars are widely present with amino acids in the Cenozoic formations of Japan. | | | |
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AMINO ACIDS CONTAINED IN THE CENOZOIC FORMATIONS OF JAPAN

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I. Foreword

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The study of amino acids contained in sedimentary rocks developed rapidly following Abelson's discovery that amino acids remained in fossils which had long been treated as inorganic matter. Abelson (1954a; 1954b) detected amino acids in the fossils of Ordovician trilobites, Cretaceous and Jurassic dinosaurs, and Devonian fish by means of the paper chromatography method; at the same time, he measured the thermal decomposition rate of alanine, a kind of amino acid, and estimated the half-life of alanine, when it decomposed (decarbonated) at room temperature, to be approximately 2,000,000,000 years. Abelson's report was followed by successive publications on experimental research on the thermal stability of amino acids (Conway et al., 1958), and on studies of amino acids contained in fossils and sedimentary rocks (Erdman et al., 1956; Abelson, 1957, 1959; Barghoon, 1957; Ijiri et al., 1958). By now, the presence of amino acids has been reported in various sedimentary rocks of different geological periods and sedimentary environments, and even in meteorites (Kaplan et al., 1963; Degens, 1965; Manskaya, 1968; Swain, 1970).

In Japan, Ijiri and Fujiwara first developed the field of amino acids in paleontology. Ijiri and Fujiwara detected ten kinds of amino acids from the molar fossils of Stegodon akashiensis and Elephas primigenius and established that these amino acids originated in the collagen tissues which remained in the molar fossils; they proved experimentally that the collagen tissues remaining in the molar fossils of Rhinoceras, Elephas primigenius,

*Numbers in the margin indicate pagination in the foreign text.

and Elephas naumanni retained the capacity to undergo calcification (to deposit hydroxyapatite) under proper conditions (Ijiri et al., 1958, 1959; Ijiri, 1959). Since then, research on amino acids remaining in fossils has been continued by Akiyama (1964) and Hotta (1965). In the field of sediments, Hoshino (1962) detected nine kinds of amino acids for the first time in blue clay from the Japan Sea Trench. Thereafter, amino acids were detected by Hashimoto (1965) in the sedimentary rocks of the Tertiary and Jurassic periods, and by Kawahara et al. (1966) in the sediments around the Japan Sea Trench. These studies proved for the first time the presence of amino acids in the Japan Sea Trench and the seabed around it, as well as in the sedimentary rocks of Japan.

The writer began her research on amino acids contained in representative geological formations of Japan with the aim of further developing these studies to the level which would enable the discussion of geological questions. Up to the present, the writer has analyzed amino acids contained in the Cenozoic formations of the Osaka region, Boso Peninsula, Niigata region and Joban region, and has published studies on the results and analytical methods with respect to the Osaka region and Boso Peninsula (Ichihara, 1967, 1969a, 1969b; Ichihara et al., 1968, 1969; Ichihara, 1970). In this present research, the writer conducted the analysis from Alluvial formations to successively older formations. And, having analyzed up to the sedimentary rocks of the Oligocene period, and ascertaining the tendency of the amino acid concentration to diminish with the age of the sedimentary rocks, encountered a sample from which no amino acid could be detected. The writer does not intend to conclude from this that "no amino acid remains in sedimentary rocks in Japan older than the Oligocene period," but she does believe that these findings are useful for research into the conditions under which amino acids contained in sedimentary rocks in Japan decompose and disappear, accompanied by diagenesis.

The present dissertation is a summary of the characteristics of the amino acids contained in the Cenozoic formations of Japan on the basis of previously published results of research, together with some unpublished material.

Amino acids have been detected in the analyses of a few samples from Mesozoic and Paleozoic formations. But their concentration levels diminish as the geological age gets older, to the point where it is difficult to judge whether the amino acids detected have their origin in organisms of the past, or in contamination by present day organisms. As there has been a case of amino acids reported in the rocks of the Cambrian period, which was later shown to have been due to contamination by living organisms (lichen deposited on the rock surface) (Abelson et al., 1968), the writer would like to go on to research on Mesozoic and Paleozoic sedimentary rocks in the future with extreme caution.

II. On Amino Acids Contained in Sedimentary Rocks

Before entering the main discussion, the writer would like to mention briefly some generally accepted opinions on amino acids contained in sedimentary rocks.

Amino acids contained in sedimentary rocks have their origin in peptide, a protein synthesized by organisms. However, the chances of peptide remaining in sediments without degenerating are rare, except for cases of organisms like shells which are isolated from the outside. The remains of benthic animals which live on the surface layer of sediments, and remains of plankton which sank through the water are rapidly decomposed on the surface layer of sediments by microbes. And the protein peptide which constituted the organisms is consumed as a source of nutrition and energy of the microbes. This also applies to other organic substances composing organisms (carbohydrates, fats, etc.), so that in sediments

in which microbe activity has already ceased, it can be considered that only organic matter which is of no value to microbes, or which is hard to decompose, remains. This type of residual organic matter is called humin, and it is a complex condensation product of amino acids, fatty acids, sugar, pigments, lignin, etc., which has no fixed chemical formula and is scattered in the sediment as an organic colloid. Consequently, amino acids detected in sedimentary rocks are what used to constitute a part of organic colloids called humin, kerogen, etc., and, with the exception of special cases, did not exist in the state of free amino acids, free peptide, or free proteins.¹

Since the density of bacteria found in seabed mud exceed $1 \cdot 10^8$ per gram of sediment at the surface, but decreases to $2 \cdot 10^3 - 3 \cdot 10^3$ at the depths of 50 to 100 cm (Degens, 1967), it is probably feasible to consider the depth at which decomposition by microbes occurs to be geologically quite shallow.

III. Samples, Analysis Method

The samples used in this study were mainly muddy sediments (50 samples including 2 samples of sandy sediments), which were collected in the four regions of Osaka, Boso, Niigata and Joban. The names of formations, the geological periods, absolute ages,² etc., are indicated in Table 1. Fresh portions of the samples were dessicated until they were in powder form, and then used for analysis. /71

The analysis method used in this research is based on 1) hydrolysis of the sample with hydrochloric acid, 2) desalination of the hydrolysis solution with ion-exchanging resin, and 3) separation of

¹A large quantity of free amino acids is detected from reductive sediments of the present day, and sometimes peptide and proteins remain in organic remnants in deserts and places permeated by asphalt (Degens, 1967).

²The absolute ages of samples are based on Minoru Ichihara et al. (1960, 1970), Ikebe et al. (1969), and Berggren (1969).

TABLE 1. TABLE SHOWING REGIONS OF COLLECTION, NAMES OF FORMATIONS, GEOLOGICAL PERIODS, AMINO ACID CONCENTRATIONS, ETC.

| Region of Collection | Formation | Geological Period | Absolute Age 10 ⁶ years | Sedimentary Water | Sample Collected | Analysis Frequency | Amino Acid Concentration, g/g* |
|----------------------|-------------------------------|--------------------------------|------------------------------------|----------------------------|------------------|--------------------|--------------------------------|
| Osaka region | Osaka Alluvial silt formation | Alluvial | <0.01 | Steam, sea water | Silty B | 2 | 900, 1700 |
| | Umeda clay form. | Alluvial | | Sea water (basin) | Clayey B | 5 | 1000-1800 |
| | basal sand form. | Alluvial | | Steam | Silty, sandy B | 2 | 100-300 |
| | Osaka formation group | Late Pliocene - early Diluvian | 0.4-3 | Steam, fresh and sea water | Clayey, silty B | 19 | 10-520 |
| Boso peninsula | Sagami form. group | Higashiyatsu form. | ±0.3 | Sea water (shallow) | Clayey (outcrop) | 1 | 60 |
| | | Sanuki formation | 0.5 | Sea water (shallow) | Muddy (outcrop) | 1 | 44 |
| | | Kakinokidai form. | 0.7 | Sea (semi-deep) | Muddy (outcrop) | 1 | 36 |
| | | Kokumoto form. | 0.7-1.2 | Sea (semi-deep) | Muddy (outcrop) | 2 | 24, 29 |
| | | Umegase formation | 1.2-2.5 | Sea (semi-deep) | Muddy (outcrop) | 2 | 23, 35 |
| | | late Pliocene - early Diluvian | 2.5-3 | Sea (semi-deep) | Muddy (outcrop) | 2 | 26, 28 |
| | | Pliocene | 3-5 | Sea (semi-deep) | Muddy (outcrop) | 1 | 18 |
| | | Kiwada formation | | | | | |
| | | Anno formation | 7-10 | Sea water | Muddy (outcrop) | 1 | 5 |
| | | Kiyosumi form. | 10-12 | Sea water | Muddy (outcrop) | 1 | 15 |
| | | Amatsu formation | 12-15 | Sea water | Muddy (outcrop) | 2 | 5, 9 |
| | | Kinone formation | 15-18 | Sea water | Muddy (outcrop) | 1 | 9 |
| | Hota formation group | Miocene | ±20 | Sea water | Muddy (outcrop) | 1 | 15 |
| Niigata region | Uonuma form. group | Tsukayama form. | 1-2 | Steam, fresh (shallow) | Muddy (outcrop) | 1 | 44 |
| | | Haizume form. | 3-5 | Sea water (shallow) | Muddy (outcrop) | 1 | 16 |
| | Nakagoe form. gr. | Hamachu form. | <5-<10 | Sea water | Muddy (outcrop) | 1 | 3 |
| | Keijo form. group | Shichiya form. | 15-20 | Sea water | Mumus mud B | 1 | 4 |
| Joban region | Hakusui form. gr. | Shirasaka form. | ±25 | Steam, sea (shallow) | Muddy B | 1 | 0 |
| | | Ishigikyo coalbed | ±30 | Fresh water | Muddy B | 1 | 0 |

*Leucine equivalent.

B: Boring core.

amino acids by the paper chromatography method. Since the actual procedure has already been reported (Ichihara, 1969a), its explanation will be omitted here.

IV. Amino Acid Concentration

The amino acid concentrations of 50 samples obtained from Cenozoic formations of Japan were measured and the following results were obtained.

A. Alluvial Terrane (Holocene Terrane, 0-0.01·10⁶ years)

The amino acid concentration levels of the Alluvial terrane were determined from the core samples of boring conducted in Osaka (Ichihara, 1967). The results are shown in Fig. 1 and Table 1. As can be gathered from the figure, the amino acid concentration decreases from the top to the bottom of the boring core. However, the decrease from the upper part to the lower part is, strictly speaking, recognized in the clay layer (the Umeda clay formation at the depths of 15 to 23 m), and the concentration in the silt layer just above the clay layer is less than the concentration in the upper part of the clay layer. This demonstrates the fact that amino acid concentration is related not only to depth but also to the grain size of sediments -- the amino acid concentration in coarse grain sediments is less than that in fine grain sediments.

As reasons for the high concentration of amino acids in clay layers, the adsorption capacity of clay minerals and the reduction aspect of the sedimentary environment may be considered. The fact that clay minerals, especially montmorillonite, adsorb amino acids has been confirmed in experiments by Bada et al. (1960) and Stevenson et al. (1970). However, from the writer's experience in separating clay minerals from the clay in the Osaka Alluvial formation (Ichihara, 1960), the portion in the clay from this formation, which can be termed clay mineral minerologically, is extremely small. Consequently, it seems inappropriate to seek the cause for the

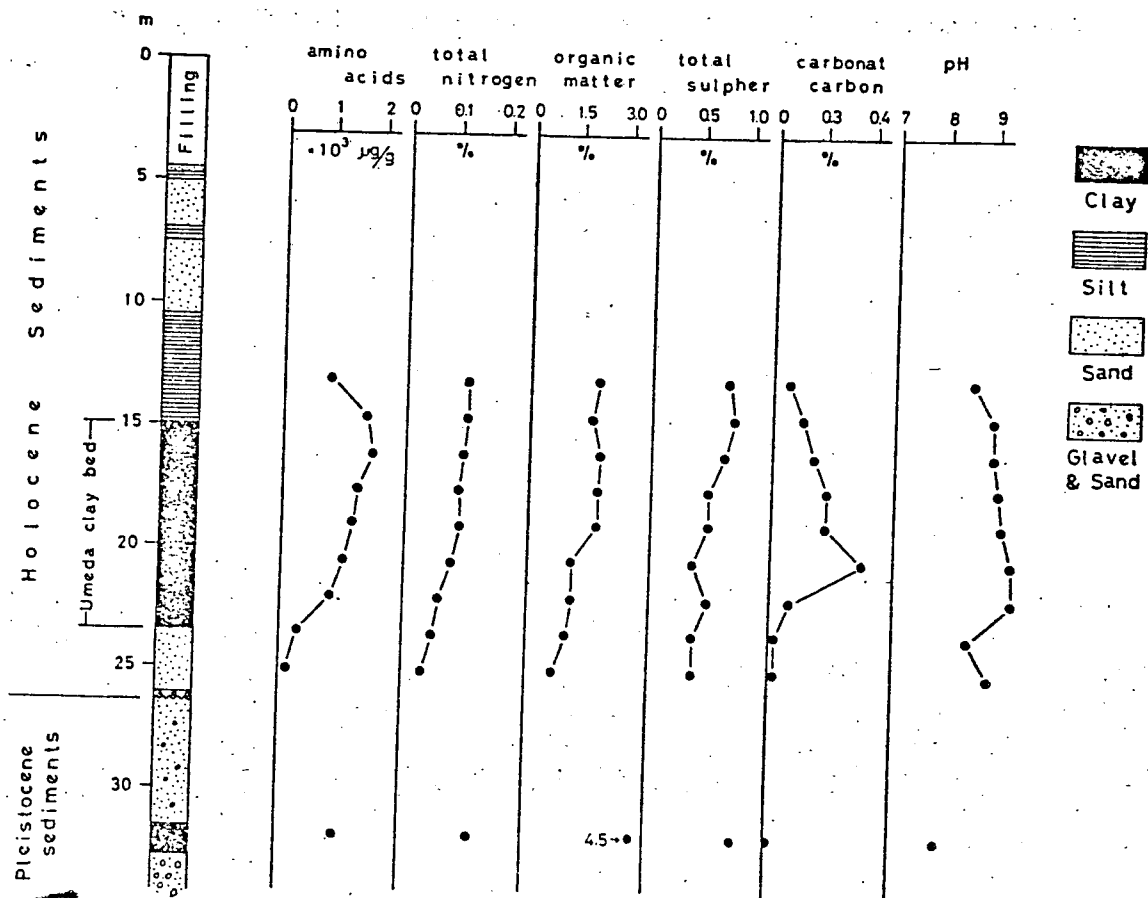


Fig. 1. Diagram showing the concentration levels of amino acids and other substances in the Osaka Alluvial formation (addition and revision to Ichihara, 1967).

higher amino acid concentration in clay layers compared to silt layers in the adsorption capacity of clay minerals in the case of the Osaka Alluvial formation. It is more valid to consider the reductive nature of the sedimentary environment. The environment in which clay sediments accumulate is a body of water in which the current is mild, and, with the exception of deep seabeds, it is generally deficient in oxygen. As microbe activity is low there, the decomposition of amino compounds and other organic matter composing organisms is rather slow. The fact that the Umeda clay formation shows higher amino acid concentration than the silt layer above it is probably due to sedimentation in such a reductive environment. The fact that the Umeda clay formation consists of sediments in reductive condition is also demonstrated by the

concentration level of shell fossils³ (Ichihara, M., 1954; Kinki Group, Society for Comprehensive study of the Quaternary Era, 1969) and of sulfur (Ichihara, 1967).

Amino acid concentration levels in Alluvial terrane reported abroad include values of 3500 μg from the boring core collected in the seabed (depth, 23 m) southeast of Devon in England (muddy and peaty samples, sample collection depth 175 cm, geological period 8580 y. B.P.) (Clarke, 1967), and approximately 400 μg from the boring core collected from the continental shelf in the Gulf of Mexico (muddy sample, sample collection depth 120 cm, geological period 2000 to 3000 years) (Erdman et al., 1956). As the value for the Umeda clay formation of the Osaka Alluvial terrane is 1000 to 1800 $\mu\text{g/g}$ (leucine equivalent, as in all concentration levels mentioned from here on), it is believed that the amino acid concentration of the Alluvial terrane must show various values depending on sedimentary conditions.

B. Diluvian Terrane (Pleistocene Terrane, $0.01-2 \cdot 10^6$ years), Pliocene Terrane ($2-6 \cdot 10^6$ years)

The amino acid concentration levels for the Diluvian and Pliocene terranes were measured in samples collected from the Osaka formation group in the Osaka region, the Sagami and Kazusa formation groups in the Boso Peninsula, and the Uonuma and Nakagoe formation groups in the Niigata region. The 19 samples from the Osaka formation group (late Pliocene period - early Diluvian period) are muddy samples collected from the core of boring (OD-5) conducted at the depth of 700 m at Tonouchi in Amagasaki City. The result of the measurement of the amino acid concentration levels of these samples is shown in Fig. 2 and Table 1 (Ichihara et al., 1968). As

³Shell fossils such as Theora lubrica and Raeta pulchella, which are index species of the Gonai Mud Belt (reductive environment), are found in the Umeda clay formation.

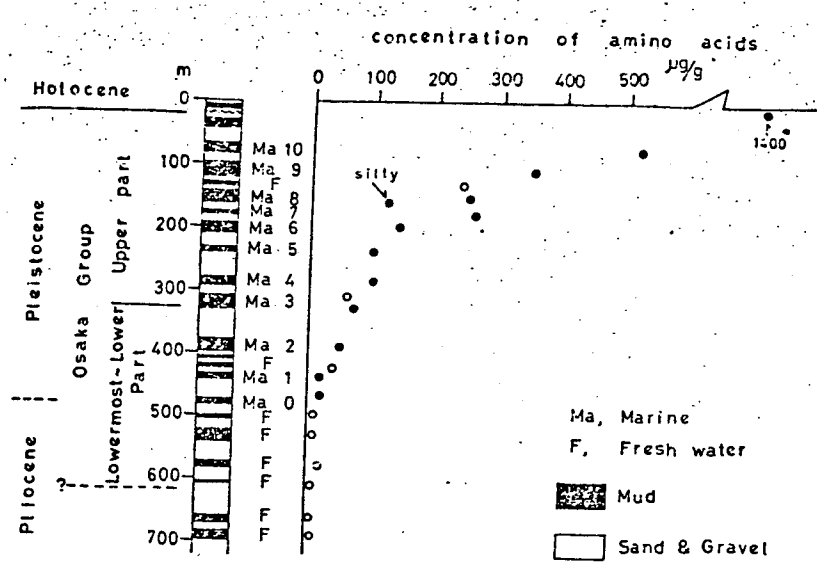


Fig. 2. Diagram showing the amino acid concentration levels in the muddy sediments from the Osaka formation group of the Osaka Alluvial terrane (addition and revision to Ichihara et al., 1968).

*Average for Umeda clay formation.

can be gathered from the diagram, the amino acid concentration levels from layer Ma 10 to layer Ma 1 and layer Ma 0 (early Diluvian) of the Osaka formation group decrease exponentially with the increase in burial depth. The amino acid concentration below layer Ma 0 (late Pliocene) shows an almost uniform value (10, 20 $\mu\text{g/g}$), and does not show a tendency to diminish.

The Osaka formation group consists of limnological and marine sediments,⁴ and the marine sediments are only seen above layer Ma 0 (Minoru Ichihara, 1960, 1966). The samples analyzed include 12 samples of marine sediments and seven samples of limnological sediments, but, as is clear from Fig. 2, no difference is observed in the amino acid concentration levels between the limnological sediments and marine sediments. On the other hand, a case of the amino acid concentration varying according to grain size was observed. When the amino acid concentration levels of a clayey sample and a silty sample from layer Ma 8 are compared, the amino acid

⁴Mainly produced in the inner bay, and including those produced in steam.

concentration of the silty sample is less than one half that of the clayey sample.

The writer would next like to examine the relationship between the burial depth and the amino acid concentration of the samples. It has already been mentioned that the amino acid concentration level decreased exponentially with respect to the burial depth from layer Ma 10 to around layer Ma 1 in the Osaka formation group. A similar relationship has been recognized in the boring core collected at the depth of 170 m off the California coast (water depth, 3566 m) (experimental Mohole drilling, Alluvial period - Miocene period) (Rittenberg et al., 1963; Degens, 1964a). When analytical values (excluding those of silty and sandy sediments) from the Osaka boring core samples (Osaka formation group, Osaka Alluvial terrane) and the analytical values reported from the boring core samples from offshore California are plotted on the same graph, Fig. 3 is obtained. As it can be seen from this graph, the loga-

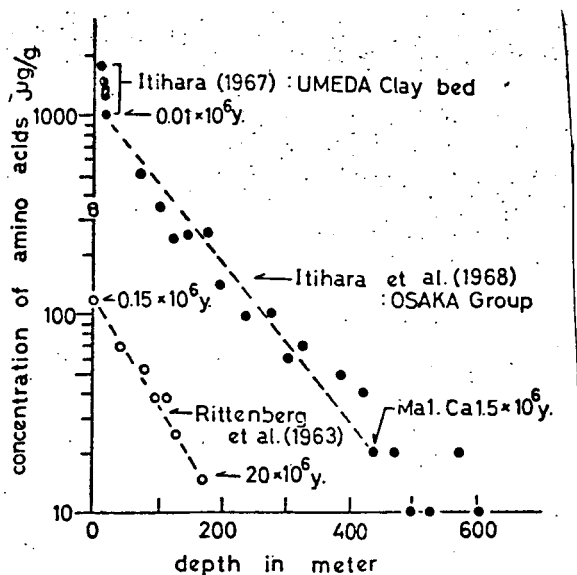


Fig. 3. Diagram showing the relationship between amino acid concentration and burial depth and absolute age.

rithmic values of the amino acid concentration decrease in an almost linear relationship with respect to the burial depth from the basal part of the Umeda clay formation to layer Ma 1 of the Osaka formation group in the case of Osaka, and lower than 1.5 m below the seabed surface in the case of offshore California. The decrease in amino acid concentration seen down to 22 m below earth surface /73 in Osaka (basal part of the Umeda clay formation) and down to 1.5 m below the seabed surface offshore

in California is respectively sharper compared to further down. The writer believes that down to these depths, some form of microbe

activity may be affecting the rate of decomposition of the amino acids. Now, taking the straight line segment⁵ excluding the above parts, if the constant a for the decomposition rate is sought from the equation $\log y = \log b - ax$ by substituting the burial depth with the estimated absolute age of the sample, 1.14 is obtained for the Osaka boring core and 0.05 for the California offshore boring core. However, y is the amino acid concentration with respect to the particular period, and $\mu\text{g/g}$ and b are the amino acid concentration, at the time when rapid decomposition following sedimentation has ended, so that in the case of Osaka, the value at 22 m below earth surface (basal part of the Umeda clay formation, $0.01 \cdot 10^6$ years), 1000 $\mu\text{g/g}$, and in the case of offshore California, the value at 1.5 m below the seabed surface (approximately $0.15 \cdot 10^6$ years),⁶ 116 $\mu\text{g/g}$, are taken as the concentration levels for their respective periods. With x as the absolute age measured from the time the rapid decomposition ended (its unit is $1 \cdot 10^6$ year), in Osaka, the absolute age of the sample obtained from layer Ma 1 at the depth of about 440 m was assumed to be $1.5 - 0.01 \cdot 10^6$ year, and for California offshore, the absolute age of the sample from the lowest layer was assumed to be $20 - 0.15 \cdot 10^6$ year.⁷ The a values obtained here show that the amino acid decomposition rate of the Inner Bay reductive sediments, represented by the Osaka Diluvian group, is faster than the amino acid decomposition rate of the deep sea oxidative sediments, represented by the offshore California boring core.

The amino acid concentration levels of muddy samples from the Sagami formation group (middle and late Diluvian) and Kazusa formation group (Pliocene - early Diluvian) of Boso Peninsula, and from

⁵Segment shown by a broken line in Fig. 3.

⁶Calculated from sedimentation rate of 1 cm / 1000 y (Degens, 1965).

⁷As Degens uses $15 \cdot 10^6$ y as the age covered by the core in "Geochemistry of Sediment," and $20 \cdot 10^6$ y in Geochim. et Cosmochim. (1964a), the writer took the median and used $20 \cdot 10^6$ y.

the Uonuma formation group (Diluvian) and the Nakagoe formation group (Pliocene) also diminish as the formations get older (Ichihara et al., 1969) (Table 1 and Fig. 4). The amino acid concentration levels of these samples were compared with the amino acid concentration levels of the muddy samples from the Osaka formation group to examine whether there were any regional characteristics.

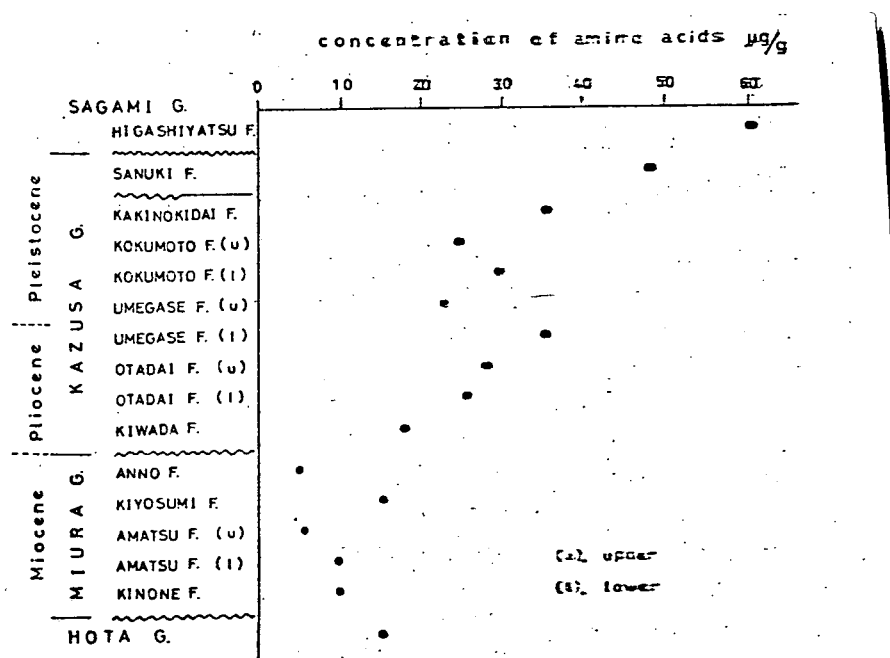


Fig. 4. Diagram showing the amino acid concentration levels in the Cenozoic formations of Boso Peninsula (Ichihara et al., 1969).

When the amino acid concentration levels of the Pliocene terrane from the three regions of Boso, Niigata and Osaka are compared, the level is 18-35 µg/g for the Kazusa formation group, 3 µg/g and 16 µg/g for the Nakagoe formation group, and 10 µg/g and 20 µg/g for the Osaka formation group, showing the quantity of amino acids contained in the Pliocene terrane of these regions to be about equal. As the Pliocene terrane in the Osaka region (formation lower than layer Ma 0 of the Osaka formation group) consists of limnological sediments, the Pliocene terrane in Boso Peninsula (lower part of Umegase formation, Otadai formation and Kiwada formation) consists of semi-deep sea sediments, and the Pliocene terrane in the Niigata

region (Haizume and Hamachu formations) consists of shallow sea sediments from the Japan Sea coastal oilfield region, each sedimentary environment is different, but this difference in the sedimentary environment is hardly reflected in the amino acid concentration levels.

When the amino acid concentration levels of the Diluvian terrane are compared, the amino acid content is greater in the Osaka formation group than in the Kazusa formation group, and the difference in the concentration levels becomes greater as the formation gets closer to the top. For example, the amino acid concentration levels in the top part of the Umegase formation and in the Kokumoto formation are, respectively, 23 $\mu\text{g/g}$ and 29 $\mu\text{g/g}$, and since it is 20-70 $\mu\text{g/g}$ in the lower part of the Osaka formation group (layer Ma 3 to layer Ma 0), which is believed to correspond approximately to these formation levels, the difference in the amino acid concentration between the Kazusa formation group and the Osaka formation group around this formation level is not so great. However, the amino acid concentration of 44 $\mu\text{g/g}$ in the Sanuki formation, which is comparable to the upper part of the Osaka formation group (above layer Ma 3), is obviously much lower than the 100 $\mu\text{g/g}$ of layer Ma 4 and 520 of layer Ma 10 of the Osaka formation group. Furthermore, the amino acid concentration of 60 $\mu\text{g/g}$ shown by the Higashiyatsu formation of the Sagami formation group (middle Diluvian) is considerably lower than the quantity expected from the values of the Osaka formation group or the Osaka Alluvial formation. Also, the amino acid concentration of the lower part of the Uonuma formation group (middle part of the Tsukayama formation) in the Niigata region is 44 $\mu\text{g/g}$, a concentration level which corresponds roughly to the value for layer Ma 2 of the Osaka formation group. As reasons for the amino acid concentration levels in the Diluvian terrane of Boso Peninsula, especially in the Sanuki and Higashiyatsu formations, being much lower than values expected from the concentration levels in the

Osaka Alluvial formation and the Osaka formation group, differences in the sedimentary environment and in the weathering process may be considered. The writer attaches importance to the fact that samples collected in Boso Peninsula were from outcrop; and in the case of the Higashiyatsu and Sanuki formations which were not yet in the advanced stage of consolidation, the writer deduces that the decomposition and disappearance of amino acids contained in parts close to the surface may be rapid due to the permeation of rain water and the supply of oxygen (Ichihara et al., 1969).

C. Miocene Terrane (6-23 · 10⁶ years)

The amino acid concentration levels of the Miocene terrane were measured in the samples collected from the Miura and Hota formation groups in Boso Peninsula and the Keijo formation group in Niigata (Fig. 4, Table 1). The amino acid concentrations of the Miura and Hota formation groups is 5-15 µg/g, and the concentration of the Keijo formation group (Shichiya formation) is 4 µg/g. The Shichiya formation consists of black shale, a parent rock of petroleum. The Shichiya formation samples, which have lower amino acid concentration compared to Miura and Hota formation groups, were collected from the boring core. The boring was conducted in the oilfield region of Niigata. The collection depth was 4755 m. If the subterranean heat increase rate is 1°C / 50 m, they must have been placed in the condition of almost 100°C. The low amino acid concentration of these samples is perhaps the result of the acceleration of decomposition by subterranean heat.

The geological period of the lowermost sample in the boring conducted off the coast of California is Miocene (Rittenberg et al., 1963), and its amino acid concentration is 15 µg/g. This value agrees well with the Miocene group from Boso Peninsula. Also, Jones et al. (1960) report that amino acids are not detected in coniferous lignite of the Miocene period collected from Libber mine in Germany.

D. Oligocene Terrane (23-36 · 10⁶ years)

The amino acid concentration for the Oligocene terrane was measured in two samples collected from the Shirasaka formation and the Ishigikyo coalbed of the Hakusui formation group distributed in the Joban region. Both samples were collected from boring cores, and collection depth was 500 m below earth surface for the Shirasaka formation and 1240 m below sea level for the Ishigikyo coalbed. Amino acids could not be detected in these two samples. The Shirasaka formation sample is grey mudstone and the Ishigikyo sample is grey-white mudstone containing sandy parts. It appears that when the geological period goes as far back as the Oligocene period, there are only a few sedimentary rocks in which amino acids remain.

Erdman (1956) collected black shale samples of the Oligocene period from the core of boring conducted in Texas (5000 ft, about 30·10⁶ y B.P.), and reported the detection of 0.51 μmol/g (67 μg/g, leucine equivalent) of amino acids. This concentration level corresponds in Japan to layer Ma 3 of the Osaka formation group, and is a value that can not be expected of the Oligocene group in Japan.

E. Residual Quantity of Amino Acids and Geological Terrane

When 2 sandy samples (Alluvial formation) are removed from the 50 samples collected from the Cenozoic formations of Japan, and the amino acid concentration levels of the remaining 48 samples are represented graphically according to their geological terranes, Fig. 5 is obtained. The quantity of amino acids contained in 1 g of sediment is 900 to 1800 μg for the Alluvial terrane, 20 to 520 μg for the Diluvian terrane (early - middle), 3 to 35 μg for the Pliocene terrane, 4 to 15 μg for the Miocene terrane, and 0 μg for the Oligocene terrane. Since there is no analytical value for 75 samples⁸ which belong positively to the late Diluvian terrane,

⁸The concentration analyzed by the writer in the clay of the so-

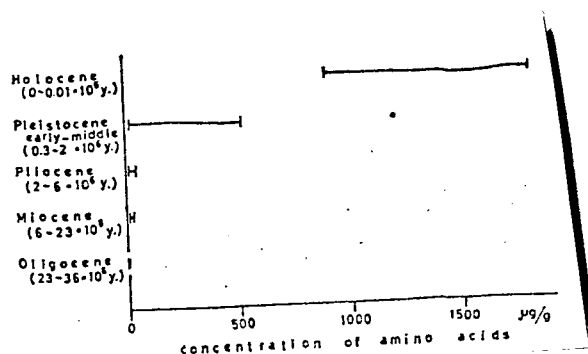


Fig. 5. Diagram showing the concentration of amino acids contained in the Cenozoic formations of Japan. (For *, see text and footnote 8.)

there is a gap between the amino acid concentrations for the Alluvial terrane and the Diluvian terrane. However, as it can be seen in the diagram, amino acids contained in muddy sediments of the Cenozoic formations of Japan disappear for the most part in the Alluvial and Diluvian terranes, remain in small

quantity in the Pliocene and Miocene terranes, and are no longer detectable in the Oligocene terrane. Although it can not be concluded that no amino acid remains in sedimentary rocks earlier than the Oligocene period in Japan solely on the basis of analytical values mentioned here, if amino acids are detected in sedimentary rocks earlier than the Oligocene period, the question of whether such amino acids may not be due to contamination by living organisms must be thoroughly examined.

V. Amino Acid Compositions

Of the 50 samples whose amino acid concentration levels were measured, 40 samples were examined for their amino acid composition. The details have already been reported (Ichihara, 1967, 1966b; Ichihara et al., 1968, 1969; Ichihara, 1970). The findings of this series of studies are as follows.

8(cont'd.) called Amamitsu formation (late Diluvian formation?), which lies beneath the Osaka Alluvial formation, was 1200 µg/g. If the Amamitsu formation is a late Diluvian formation, the gap in amino acid concentration between the Alluvial and Diluvian terranes would be filled. For reference, the concentration of the Amamitsu formation is indicated by an asterisk.

A. On the Uniformity of Amino Acid Composition

Nineteen kinds of amino acids were detected in the 40 Cenozoic samples. Of these, 17 are α -amino acids, which, with the exception of ornithine, are ordinary amino acids composing proteins (ornithine is not found in proteins, but it plays an important physiological role within organisms). The remaining two are γ -amino butyric acid and β -alanine, which are special amino acids with no amino radical in the α order, but with amino radicals attached to a γ order and β order, respectively. These are not found in proteins. The distribution of the 19 amino acids detected in the 40 samples is as follows.

- 1) Amino acids detected in all samples:
leucine, valine, γ -amino butyric acid, glycine, alanine, threonine, serine
- 2) Amino acids detected in most of the samples:
phenyl-alanine, tyrosine, β -alanine, glutamic acid, aspartic acid, arginine, histidine, lysine, ornithine
- 3) Amino acids detected in about half of the samples:
proline, hydroxyproline
- 4) Amino acid rarely detected
cystine

When the 40 samples, whose amino acid compositions were examined, are then divided into 6 groups on the basis of geological terrane and sedimentary basin (Alluvial, Diluvian and Pliocene terranes from the Osaka region; Diluvian, Pliocene and Miocene terranes from the Boso Peninsula), and the mean amino acid composition percentages are obtained, Table 2 is the result. As can be seen from the table, the amino acid composition percentages of the six groups, while there are some differences, are fairly similar. Furthermore, the differences in composition percentage among the groups are not related to the geological terrane or sedimentary basin. In spite of the fact that the amino acid concentration decreases as the geological period of the samples gets older, so that there is a

TABLE 2. TABLE SHOWING MEAN VALUES OF AMINO ACID PERCENTAGES.

| Region | Geol. Terrane | Phe | Tyr | Leu | Val | γ -ABuA | Pro | β -Ala | Ala | Thr | Gly | Ser | Hypro | Cys | Glu | Asp | Arg | His | Lys | Orn | Anal. Freq. |
|----------------|---------------|-----|-----|-----|-----|----------------|-----|--------------|-----|-----|-----|-----|-------|-----|-----|-----|-----|-----|-----|-----|-------------|
| Osaka region | Alluvial ter. | 14 | + | 6 | 4 | 3 | + | + | 13 | 6 | 19 | 6 | + | + | 7 | 3 | + | + | + | + | 9 |
| | Diluvian ter. | 6 | + | 16 | 9 | 7 | + | 5 | 9 | 3 | 7 | 2 | + | + | 4 | 5 | 7 | 8 | + | + | 13 |
| | Pliocene ter. | 13 | + | 16 | 7 | 4 | - | 4 | 8 | 3 | 10 | 3 | + | - | 3 | 5 | 7 | 6 | + | + | 3 |
| Boso Peninsula | Diluvian ter. | 6 | 4 | 17 | 10 | 5 | + | 5 | 10 | 5 | 10 | 4 | + | - | 1 | 3 | 1 | 2 | 10 | 4 | 6 |
| | Pliocene ter. | 15 | + | 14 | 10 | 8 | + | 5 | 7 | 2 | 12 | 6 | + | - | 2 | + | + | + | 13 | 4 | 4 |
| | Miocene ter. | 16 | 3 | 11 | 10 | 3 | + | 2 | 10 | 9 | 5 | 10 | - | - | + | ? | 3 | + | 11 | 5 | 5 |

Phe: phenyl-alanine, Tyr: tyrosine, Leu: leucine, Val: valine, γ -ABuA: γ -amino butyric acid, Pro: proline, β -Ala: β -alanine, Ala: alanine, Thr: threonine, Gly: glycine, Ser: serine, Hypro: hydroxyproline, Cys: cystine, Glu: glutamic acid, Asp: aspartic acid, Arg: arginine, His: histidine, Lys: lysine, Orn: ornithine

difference of almost 100 times between an Alluvial sample and a Miocene sample, the amino acid composition is about the same in the samples of all the geological periods. This fact signifies that the 19 kinds of amino acids decrease at a uniform rate with the passage of geological ages. Since it has been proven that the decomposition rate of free amino acids varies according to the kind of amino acid (the reaction rate constants in hydrolysis are different) (Abelson, 1959; Vallentine, 1964), if the amino acids contained in sedimentary rocks are free amino acids, they should obviously disappear successively starting with amino acids of fast decomposition rate, so that it would be inconceivable that the composition of the amino acids should remain unchanged with the passage of geological ages. /76

The uniformity of the amino acid composition percentages seen in Table 2 probably indicates that the amino acids contained in sedimentary rocks are not in a free state, but are part of complex organic matter called humin or kerogen, so that the unstable amino

acids which should disappear if they were in a free state are protected structurally and remain intact.

Degens (1964b) reported that a large quantity of serine, an amino acid classified as unstable by Abelson,⁹ was contained in salt water from a Tertiary Era oilfield, and supposed that the stability with respect to diagenesis differed between free amino acids and bound amino acids. And he established his own order of amino acid stability with respect to diagenesis (Degens, 1965). On the other hand, Jones et al. (1960) have written that a large majority of amino acids remaining in shale from the Eocene period were those classified by Abelson to be thermally stable.⁹ The analytical results obtained by the writer agree with Degens' results with respect to the fact that serine and threonine, which are thermally unstable amino acids, were positively identified in all the samples. However, as far as the writer could determine through the analysis of Cenozoic samples, it was not possible to formulate an order in the stability of amino acids with respect to diagenesis.

B. On γ -Amino Butyric Acid and β -Alanine

γ -amino butyric acid and β -alanine are detected from Cenozoic formations of Japan, as well as α -amino acids.

γ -amino butyric acid was detected without exception from the samples analyzed, and its quantity comprises 3-8% of the total quantity of amino acids (Table 2). In one-dimensional paper chromatograph development with acetic acid, butanol and water as solvents, this amino acid yields an Rf value which is lower than that of valine and higher than those of oxyproline and alanine. This amino acid was discovered in the rhizome of potato plants, and later

⁹Abelson (1959) classified amino acids in the following manner on the basis of their thermal stability:

Relatively stable: Ala, Gly, Glu, Leu, Isoleu, Pro, Val.

Moderately stable: Asp, Lys, Phe.

Relatively unstable: Ser, Thr, Arg, Tyr.

detected also in decomposed yeast germ, soil and cow brain, but its biochemical function in organisms has not yet been clarified (Akabori and Mizushima, 1954). Jones and Vallentyne (1960) detected γ -amino butyric acid in Eocene shale from Colorado, and supposed that the amino acid had been contained in shale since sedimentation, but later, Vallentyne (1964) ascertained that γ -amino butyric acid was generated in the thermal decomposition of glutamic acid accompanied by diagenesis. Since this amino acid has been detected in Alluvial formations of Osaka without exception, as well as in various kinds of soils in the United States (Stevenson, 1954, 1956a, 1956b), the writer would rather consider this amino acid as a biogenetic product of the decomposition of organic remains by microbes, than a product of diagenesis.

β -alanine was detected in all samples analyzed, with the exception of the two samples collected from Miocene terrane, and comprises 2-5% of the total quantity of amino acids (Table 2). This amino acid is present in animal muscle tissues in the form of carnosin and anserine, is a component of pantothenic acid, and is an important amino acid for biology (Akabori and Mizushima, 1954). It has also been reported in soils (Stevenson, 1954, 1956a, 1956b). Degens (1964a) reported that this amino acid, which is not present in sea water, was detected in seabed mud, and stated that it was probably generated in the metabolic decarboxylation of aspartic acid which is present in large quantity in sea water. As this amino acid is also detected from the Alluvial formations of Osaka without exception, it is probably appropriate to support Degens' view.

C. On Amino Sugars

The writer, while trying to identify amino acids separated from sedimentary rock samples by means of two-dimensional paper chromatography, noticed that a ninhydrine positive spot was occasionally observed at a fixed position on the chromatogram, and investigated its nature by varying the development conditions. The writer

also deduced this substance to be a basic amino compound (Ichihara, 1969b). Subsequently, the writer applied the silver nitrate method and the Elson-Morgan method to the paper chromatogram of several samples collected from the Osaka formation group and the Kazusa formation group, and determined the ninhydrine positive substance in question to be amino sugars (Ichihara, 1970). Amino sugars have also been detected in boring cores from offshore California (Rittenberg et al., 1963) but the details have not been reported. As ninhydrine positive substances, which are thought to be amino sugars, are detected from a large majority of samples collected from the Osaka formation group, Sagami formation group and Kazusa formation group, and from part of the samples from the Miura formation group, it is supposed that amino sugars are widely present in the Cenozoic formations of Japan.

VI. Summary

This study clarifies the concentration, composition, and other aspects of amino acids present in the Cenozoic formations of Japan. The samples used for research were mainly muddy samples and were collected from representative Cenozoic formations of Japan distributed in the Osaka region, Boso Peninsula, Niigata region and Joban region. The following is a summary of the results of amino acid analysis of these samples. /77

1) The amino acid concentration diminishes as the geological age of the formations gets older. The quantity of amino acids contained in 1 g of muddy sediments is 900 to 1800 μg in the Alluvial terrane, 20 to 520 μg in Diluvian terrane, 3 to 35 μg in the Pliocene terrane, and 4 to 15 μg in the Miocene terrane. It is not possible to conclude from this that "no amino acid remains in formations prior to the Oligocene period in Japan," but these findings do indicate the conditions in which amino acids decompose and disappear accompanied by diagenesis. Also, the amino acid concentration, which diminishes rapidly from the Alluvial terrane to

Diluvian terrane, has an exponential relationship with the burial depth of the sediments, and reaches an approximately constant value in the vicinity of the boundary between the Diluvian terrane and the Pliocene terrane (Fig. 3). In the Pliocene and Miocene terranes, the diminution tendency is not clear.

2) Amino acid concentration is high in clayey sediments and low in sandy sediments. The cause for the high amino acid concentration in fine grain sediments is believed to be the inactivity of microbes accompanying the reduction characteristic of the sedimentary environment.

3) Nineteen kinds of amino acids were detected. Of these, 17 are α -amino acids, and the remaining two are γ -amino butyric acid and β -alanine. Of the 19 amino acids, 16 were detected in all or most of the samples, and the amino acid composition was similar among the samples. These 16 are leucine, valine, γ -amino butyric acid, glycine, alanine, threonine, serine, phenyl-alanine, tyrosine, β -alanine, glutamic acid, aspartic acid, arginine, histidine, lysine, and ornithine. Of the remaining three, proline and oxoproline are detected in about half of the samples, and cystine in a few of the samples.

4) The amino acid composition percentage is similar in all the samples. This indicates that the above-mentioned 19 amino acids decrease almost uniformly and independent of the decomposition rates of individual amino acids. It is believed the reason for this is that since the amino acids in sedimentary rocks comprise part of complex organic substances called humin or kerogen, even amino acids of fast decomposition rate escape decomposition because of their structure.

5) Apart from the α -amino acid, γ -amino butyric acid and β -alanine are also detected, but it is believed that these amino acids were not generated from α -amino acids through diagenesis,

but rather were produced biogenetically by the metabolic process of microbes.

6) It is supposed that amino sugars are widely present with amino acids in the Cenozoic formations of Japan.

VII. Postscript

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